Experimental Model for the Irradiation of Tissular Flaps in Small Animals

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INTRODUCTION

The current emphasis is on efficient reconstructive surgical treatment using autologous tissue or other substitute materials (prosthesis, implants). Current development directions in the area of oncological surgery are: improving the biocompatibility of the synthetic materials used (1) and increasing the use of the minimally invasive techniques in reconstructive surgery (2, 3). In order to standardize the treatment and to facilitate widespread access to this innovative treatment, reconstructive surgery, the concept of oncoplastic surgery has been recently developed. Notable results regarding the use of reconstructive surgery in oncology are currently obtained in some areas of oncologic surgery, such as breast cancer, especially through the use of biocompatible synthetic materials (2, 4, 5). The reconstructive surgery techniques allow a decrease in the morbidity rate and an increase of the patients’ quality of life after cervical, thoracic, pelvic or limb surgery (6, 7, 8, 9).

There is no common point of view regarding the method of applying reconstructive methods as part of the multidisciplinary treatment of oncologic patients (10). A special focus is placed on the treatment of cancer and...
on establishing certain protocols which should define the sequence of various therapeutic modalities (surgery, radiotherapy, chemotherapy and immunotherapy) (10, 11). Of great importance is the relationship between surgical treatment and radiotherapy as it is known that radiotherapy leads to deep tissue changes with negative effects on reconstruction techniques, especially when synthetic materials are used. Experimental studies on radiation effects on tissue flaps and on biocompatible materials are justified by the serious problems that occur in reconstructive surgery performed in areas affected by radiotherapy. These will offer information which can be applied in clinical practice, concerning the correct timing of reconstructive surgery within the management of the cancer patient (12-15).

Biocompatibility improvement of the implantable products is a cutting edge research subject at an international level (12, 15, 16). A synthetic product with perfect biocompatibility has not been invented yet. Experimental research regarding the biocompatibility of synthetic materials is attempting to create the future possibility of using high quality materials produced locally. Currently, the materials used are imported, thus they are of high quality but very expensive.

The present study is a comparative, prospective study concerning the biocompatibility and the behaviour to irradiation of some implants – commercially available titanium clips and pieces of silicone and polypropylene mesh - implanted in small laboratory animals. These materials have been chosen in accordance with their suitability for reconstructive surgery. The tissular reaction to radiation in the presence of implanted materials was studied in order to establish an experimental model for the irradiation of tissular flaps in small animals.

**MATERIALS AND METHOD**

The tolerance to irradiation of fasciocutaneous and muscular flaps and the tissular reaction to radiation in the presence of implanted materials (titanium clips, pieces of silicone and polypropylene mesh) were studied on a small animal model (rat). A number of 31 Wistar-Bratislava rats from the Biobasis of the Iuliu Hatieganu University of Medicine and Pharmacy Cluj-Napoca, weighting between 175-240 g (mean: 186 g), were utilized.

All research was conducted according to the *International Guiding Principles for Biomedical Research Involving Animals*, developed by the Council for International Organizations of Medical Sciences as a result of extensive international and interdisciplinary consultation (http://www.cioms.ch/publications/guidelines/1985_texts_of_guidelines.htm) and to the Directive 86/609/EEC on the protection of Animals used for Experimental and other scientific purposes (http://ec.europa.eu/food/fs/aw/aw_legislation/scientific/86-609-eeec_en.pdf)

Surgery was performed in aseptic conditions, using ordinary and microsurgical instruments, sterilized by autoclaving (135°C, 10 minutes and 2 atmospheres). For a higher dissection precision, operatory microscopes (Leica® M651, Leica Microsystems AG Switzerland) and prismatic binocular magnifying glasses (Zeiss 3.3X, focal distance 450mm) were utilized. Commercially available surgical titan clips (SLS-Clip W6060.1 micro, Vitalitec International, France, USA), pieces of silicone and modified polypropylene mesh (obtained by a new method in the Department of Materials Science and Technology, Faculty of Materials Science and Engineering, Technical University Cluj-Napoca) were used.

**Surgery**

Abdominal skin was prepared with antiseptic solution (Betadina® 10% - Povidone Iodine 10%, Egis Pharmaceutical LTD - Hungary). The inguinal flap was marked on the skin (Fig. 1), including the last nipple. The flap was dissected and the vascular pedicle was isolated (Fig. 2).

The vascular pedicles of the flaps were clamped for one hour using an atraumatic microvascular clamp, in order to simulate the ischemia phase encountered during free flap transfer.

The right rectus abdominis muscle sheet was opened and 2 normal titanium clips were inserted into the muscle, at 1 cm distance one to each other. A piece of silicone or a piece of polypropylene mesh were placed in a subcutaneous pocket at the level of the right iliac fossa.

![Fig. 1. Skin projection of the inguinal (1) and abdominal rectus (2) flaps.](image-url)
The left abdominis rectus muscle flap was repositioned and both rectus sheets were closed. The inguinal flap was repositioned and the skin was closed using 3/0 or 4/0 monofilament polypropylene.

**Radiotherapy**

A group of 5 rats was kept as control until the end of the experiment, whilst the other 20 animals (divided into 4 groups) received postoperative radiotherapy. In order to elaborate the technical protocol of irradiation, a CT scan planning was used (Fig. 3).

Considering the small irradiation fields and depth, small energy electron beam irradiation was considered more appropriate. Dosimetry tests were performed in the radiation beam to define the isodoses, the depth of the maximum dose, the path of the electrons in the tissue and the thickness of the bolus. The measurements were done in the Physics Department of the Laboratory of Radiotherapy with High Energies and Brachitherapy of the Oncological Institute, Cluj-Napoca, using the 3D Wellhofer Scanditronix computerized dosimetry system.

The percentage depth dose for the 6MeV electron beam is represented in Fig. 4.

Analyzing the percentage depth dose curve for a 6MeV electron beam delivered using the 5x5 cm electron applicator of the Siemens Primus Clinac it was concluded that, by applying a bolus of 1 cm thickness on the treated surface, a homogenous irradiated volume is obtained between the skin surface and the depth of 1 cm (Fig. 5). The dose received at 2 cm depth is under 15% from the maximum dose, giving a good protection of the internal organs. The analysis of the isodoses shows a very good framing of the area of interest (between the skin and the depth of 1 cm) in the 90% isodose, without hot spots.

Using 6MeV energy electrons produced by the Siemens Primus Clinac linear accelerator, source to surface distance (SSD)100cm, 25Gy/5fractions/5days were delivered with an 5 cm diameter applicator. Irradiation started 3 days postoperatively. A 1 cm thickness bolus was used (fig 6).

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**Fig. 2.** Vascularization of the inguinal flap: 1 – vascular pedicle of the inguinal flap; 2 – superficial femoral vessels

**Fig. 3.** CT scan delineation for the inguinal irradiation fields.

**Fig. 4.** Depth-dose curve for 6MeV beam generated by Siemens Primus Clinac, with 5x5 cm circular applicator.

**Fig. 5.** Isodose curves in irradiated volume, 6MeV beam generated by Siemens Primus Clinac with 5x5 cm circular applicator.
Microscopically, a mild inflammatory reaction with cutaneous and subcutaneous necrotic areas was noted 7 days postoperatively. At 20 days after surgery, an important proliferation of the connective tissue was noted. The muscular tissue containing the two metallic implants appeared more fibrotic.

**Samples prelevation and processing**

In days 7, 11, 15 and 20 after surgery, groups of 5 animals were sacrificed by intracardiac injection of potassium chloride 10%, under profound anaesthesia with ether.

The macroscopic aspect of the inguinal flaps was evaluated and the flaps were prelevated “en bloc” with the surrounding tissues.

The right rectus abdominis muscle sheet with the metallic implants was also harvested (figs. 5-7). The metallic clips and the other implanted materials were retrieved together with the surrounding tissues. For minimizing the tissue trauma, all the dissections were performed under operatory microscopes and using microsurgical instruments. Under optical magnification also, the tissue fragments containing the clips were cut with a scalpel blade grazing the metallic implant. The tissue samples were then marked with nylon 8/0 threads for identification of the clips’ position and preserved in formol 10%. Finally, slides for histologic analysis were prepared from all tissue samples. These were stained with hematoxillin-eosin and examined in light microscopy.

**RESULTS**

Six deaths occurred postoperatively. The evolution of the transplanted tissue in the 25 surviving rats was monitored macroscopically as well as by histological evaluation (microscopy, neovascularisation, vascular structural changes) after the prelevation of flaps in days 7, 11, 15 and 20 days after surgery.

Macroscopically, the local appearance was variable, from the complete integration of the flap into the tissular defect (Fig.7) to the complete necrosis of the fasciocutaneous and muscular flap (Fig. 8). In most animals an intermediary aspect was found, consisting of the partial dehiscence of sutures and some degree of marginal necrosis in the transferred flaps (fig. 9).
DISCUSSION

Biocompatibility improvement of the implantable products is a cutting edge research subject at an international level. Currently, in our country the materials used are imported. They are of high quality but also very expensive. Experimental research regarding the biocompatibility of synthetic materials is attempting to create the future possibility of using high quality materials produced locally. Before being tested in humans, the suitability of a specific biomaterial needs to be tested in animals. The local effects of an implantable device or a material on tissue are assessed by implantation procedures that introduce the material or device into tissue. The implanted tissue region is allowed to heal, then explanted and examined for macroscopic and microscopic tissue responses. The evaluation of the effects of the implants is by assessment of several macroscopic and microscopic parameters, including fibrosis, degeneration, presence of phagocytic cells, necrosis, fatty infiltration, and foreign debris.

The aim of our research was to establish an experimental model for the irradiation of tissular flaps in small animals, which could be appropriate for the study of tissue reactions and tolerance to radiations in the presence or absence of implanted materials. The materials used (commercially available titanium clips and locally produced polypropylene mesh and silicone implants) have been chosen in accordance with their suitability for reconstructive surgery. The tissue transplantation and synthetic materials implantation were performed first and then they were irradiated. The evolution of the transplanted tissue was monitored by histological microscopy, neovascularisation, vascular structural changes.

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The set-up of the technical protocol of irradiation was carefully done, using a CT-scan planning and performing dosimetric tests to define the irradiation parameters, so that a homogenous irradiated volume was obtained between the skin surface and the depth of 1 cm, with a good protection of the internal organs. The tissular changes, macro- and microscopically evaluated, were variable and depended on the type of biomaterial used.

We conclude that our experimental model is appropriate and can be used for the study of tissue reactions and tolerance to radiations in the presence or absence of implanted materials.

REFERENCES


Competing interests: The authors declare that they have no competing interests.

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